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CHOLESTEROL

By RUTH WOODS

Cholesterol is a common constituent of nearly all cells and fluids of the animal body. It is one of a large group of biologically important related compounds known as sterols. These include vitamin D and its precursors; the male and female sex hormones; the hormones of the adrenal cortex (such as cortisone); the bile acids; the cardiac glucosides (such as digitalis) and others. The full physiological significance of cholesterol is still unexplored. Its close chemical relation to these other sterols, however, has provoked many intriguing speculations, particularly the thought that it might serve as a starting material in the body for the manufacture of many of these vital compounds.

In recent years interest in cholesterol metabolism has centered increasingly on its possible role in the development of arteriosclerosis. This concept is not new, having been considered in its various aspects for nearly fifty years. Not until very recently, however, has it been possible to draw any definite conclusions as to the place of cholesterol in the complex picture of vascular disease. The story is long, often confused and filled with many contradictions. It involves many aspects of cholesterol metabolism, from its role in the diet of both man and experimental animals, to the intricacies of its synthesis in the body and its interrelation with other lipids. This review will attempt to set forth the story of cholesterol metabolism and its relation to the development of arteriosclerosis as far as it is known from the combined findings of historical and present day research.

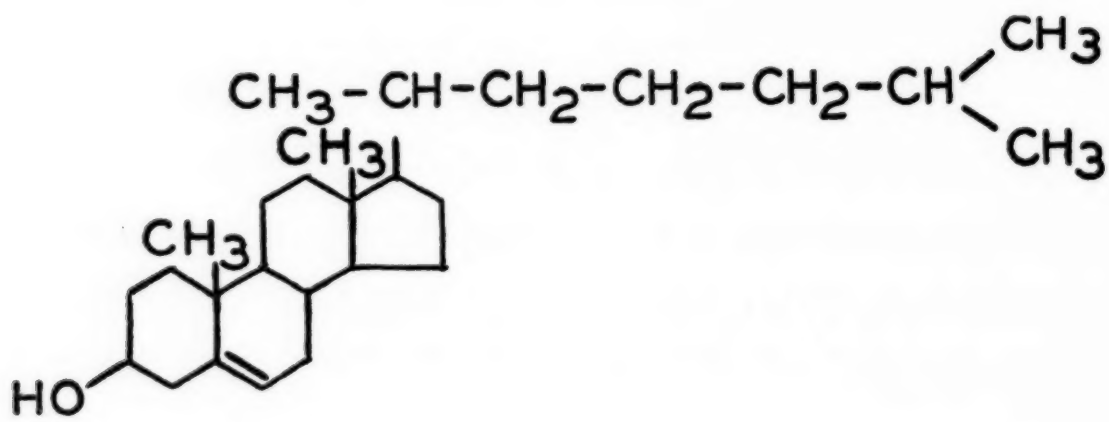
Cholesterol in the Animal Body:

Cholesterol is one of a group of closely related fat-soluble compounds known as sterols. These compounds share in common the aromatic organic structure known as the cyclyopentanoperhydrophenanthrene nucleus (see Fig. 1) and differ from each other largely by minor modifications in the nature and location of the various side chains. The sterols are widely distributed in plants and animals; cholesterol, however, occurs in animal tissues only. It is, in fact, the characteristic sterol of higher animals. Although known since the 18th century as the chief constituent of human gall stones, the formula of cholesterol was not established until after the work of Wieland and Windaus in 1928 (Fig. 1).

The relation of cholesterol to other sterols having the cyclyopentanoperhydrophenanthrene nucleus (i.e. estrogens, androgens, progesterone, vitamin D, digitalis, adrenal hormones, etc.) has been the subject of much specula-

tion. Its role in the biosynthesis of one of these compounds, *progesterone* (the hormone responsible for the maintenance of pregnancy in its early stages) has already been demonstrated (1) and the relation between the cholesterol ester reserves of the adrenals and the production of the chemically similar adrenal cortical hormones (2) suggests the possibility of cholesterol esters serving as precursors for these compounds.

Fig. 1



CHOLESTEROL, $C_{27}H_{46}O$

(a cyclyopentanoperhydrophenanthrene derivative)

Sources of Cholesterol: Cholesterol in the animal body arises from two sources. It may be absorbed* from the intestine (2a) from foods (exogenous cholesterol) or it may arise as a result of biosynthesis in the body (3) (endogenous cholesterol) from compounds as simple as water and acetate (4, 5) or others. Herbivorous animals have no source of cholesterol in their diet, but do synthesize this sterol within their bodies. Cholesterol is found in the body in two forms: Free cholesterol chiefly in brain and nerve tissue and in blood cells, and cholesterol esters (i.e. combined with fatty acids), chiefly in blood plasma and in the adrenal cortex.

Insufficient dietary cholesterol is usually balanced by increased biosynthesis. Similarly, excess dietary cholesterol is disposed of by increased excretion. The rate of disposal of cholesterol is governed by the liver and is, to some extent, limited, so that unusually excessive amounts of dietary cholesterol may accumulate in the blood (hypercholesterolemia). This disposal rate varies with individuals. It is particularly limited in herbivorous animals in which it may be relatively easy to induce experimental hypercholesterolemia.

In some herbivorous species, hypercholesterolemia is consistently followed by arteriosclerosis. In certain cases of abnormal cholesterol metabolism in man, hypercholesterolemia may be associated with arteriosclerosis, although a direct casual relationship has not been established. Cholesterol

*The cholesterol derivatives, coprosterol (excretory form) and dihydrocholesterol, are not absorbed from the intestinal tract; neither are the related plant sterols, sitosterol, stigmasterol or ergosterol (2a).

deposits have been found in certain types of sclerotic lesions of the arteries. These are some of the findings which have implicated cholesterol in the development of certain classes of arteriosclerosis and have served as a basis for research in this field.

The Nature of Arteriosclerosis:

Arteriosclerosis is generally considered to be one of the major diseases of this era. In its various forms it is responsible for more deaths and disability than any other disease. Coronary disease, cerebral hemorrhage, disturbances of the peripheral arteries and hypertension may all be traced to failure of the blood vessels due ultimately to arteriosclerosis in one form or another. As Page (6) has remarked, "The problem of arteriosclerosis is the nub of the problem of cardiovascular disease."

Literally, arteriosclerosis means a hardening of the arteries. Many types are recognized and are distinguished from each other by the nature of the degenerative changes, by the location of these changes in the arteries, and by the type and location of blood vessels involved.

Nomenclature: Because of the varied manifestations of vascular sclerosis, and the many concepts as to the exact nature and cause of the disease (often regarded as several distinct disease entities) considerable confusion in terminology has arisen. Thus, arterial lesions may occur in one or more layers of the arteries and may consist of the development of thickening fibrous tissue (hyperplasia); or of the deposition of calcium (calcinosis or sclerosis); or of abnormal deposition of cholesterol and lipids (atheromatosis); or of deposition of mucin, hyalin, and other substances. The term, *atherosclerosis,* has been suggested to indicate the combined condition of abnormal deposition of cholesterol and lipids (atheroma) and the hardening, or sclerosis, resulting from calcification. It has been impossible to find a simple, general, yet accurate term to include all manifestations of vascular lesions in terms of the various metabolic disturbances. The nearest approach has been the common, long-used name, *arteriosclerosis.* This is used to mean a thickening and hardening of the blood vessels (not only arteries, but arterioles, veins, etc.) with attendant loss of elasticity and decreased size of the passage due to a wide variety of abnormal deposits in the vascular walls. Only that phase of arteriosclerosis known as *atherosclerosis* will be discussed in this review, because this is the only condition in which abnormal cholesterol metabolism has been implicated.

The Concept of Arteriosclerosis as a Metabolic Disease: Until comparatively recently the development of arteriosclerosis was considered to be an

inevitable degenerative consequence of old age. As pointed out by Aschoff (7) some twenty years ago, "*the widespread belief that arteriosclerosis is merely a manifestation of old age does not tally with the actual facts . . . The mere aging of the vessels results in distention and dilation and consequently in increasing tortuosity. But mere aging never results in deformations of vascular wall and the vascular tube which are typical of arteriosclerosis.*" As Dragstedt (8) has further stated, "*it is a metabolic disease, slow in development and whose end results thus become manifest toward the end of life.*" This view is supported by the observation that not all old people have arteriosclerosis and that, under certain conditions, it appears in young adults and even in children.

The search for metabolic factors in the development of arteriosclerosis began with the classic studies of Anitschkow (9, 10) who in 1912 succeeded in producing lesions typical of human atherosclerosis in rabbits by feeding cholesterol dissolved in vegetable oil. The implication of cholesterol had already been foreshadowed by the findings of Windaus and of clinical investigators (11) that atherosclerotic lesions are rich in cholesterol. Anitschkow postulated the theory that "atherosclerosis is not essentially of degenerative nature, but rather of an infiltrative character (12)."

Experimental Atherosclerosis and Hypercholesterolemia:

Following the production of atherosclerosis in cholesterol-fed rabbits with lesions similar to those occurring in man, many investigators have repeated and extended these studies. Studies have involved variations in diet and have included many animal species in addition to the rabbit. Similar findings have been reported for chicks (13) and for guinea pigs (14), but attempts to reproduce these conditions in dogs, cats and monkeys were generally unsuccessful. This served as a strong argument in favor of the critics of Anitschkow who maintained that his results with rabbits were not valid because of the limitations in handling cholesterol characteristic of herbivorous animals.

In 1941, however, Steiner and Domanski (15) reported that they were able to raise the blood level of cholesterol in dogs to a moderate degree (300-400 mg. of cholesterol per 100 cc. serum) by prolonged feeding of large amounts of cholesterol-rich egg yolk powder. The hypercholesterolemia, moreover, could be maintained for a period of from 44 to 56 weeks. However, it was not followed by deposition of cholesterol leading to arteriosclerosis.* Some years later, Li and Freeman (16) also were able to produce

*It is well-known that cholesterol metabolism is influenced markedly by various hormones including, particularly, thyroxine and diethylstilbestrol. Hypercholesterolemia may occur in the

and maintain extreme hypercholesterolemia in dogs for periods up to 18 weeks (blood levels up to 1700 mg. of cholesterol per 100 cc.) by diets high in fat and low in protein. When the dietary protein was raised to adequate levels, increases in serum cholesterol were only moderate. Again, however, no signs of atherosclerosis developed, despite the severe hypercholesterolemia.

Studies with Human Subjects: Cholesterol intakes in man vary over a wide range, depending on food habits. As Keys *et al* (18a) point out, some people regularly take two eggs for breakfast, have several pats of butter at every meal and do not remove all separable fat from their twice-a-day meat. Others do not eat much milk, cheese or eggs, are sparing of butter and refuse all but lean meat. Regardless of the rest of the diet, the latter can get little more than 200-300 mg. of cholesterol per day, while the former may have at least three times this amount. Keys *et al* conducted an extensive study of several hundred normal men eating diets in this wide range of cholesterol intake. They found no influence of diet on blood cholesterol levels within this wide, normal range. They did find, however, that *complete* removal of dietary cholesterol and fat (as achieved by the Kempner rice diet†) produced a marked, rapid decline in serum cholesterol in both normal men and in patients with hypercholesterolemia. Ordinarily, however, a low cholesterol diet, permitting ordinary amounts of most foods, is far from cholesterol-free and would be ineffective in reducing blood cholesterol levels. Furthermore, a completely cholesterol-free diet containing some fat was found to result in a return of blood cholesterol to previous high levels.

Significance of Hypercholesterolemia: The feeding of large amounts of cholesterol, with or without hormones, has been shown to produce hypercholesterolemia and eventually atherosclerosis in experimental animals, both herbivorous and omnivorous. In the case of man, considerable doubt exists as to whether significant hypercholesterolemia can be induced by diet. Slight increases in blood cholesterol have been reported by Okey and Stewart (18b) who fed women four egg yolks a day for a month. Steiner and Domanski (18c) similarly caused a mild degree of hypercholesterolemia by feeding subjects 100 grams of egg yolk powder (equivalent to 12 eggs) daily for 6 to 10 weeks. Reduction of blood cholesterol levels by drastic dietary restriction has also been achieved, as already described.

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absence of sufficient thyroid control (17) or under the influence of excessive amounts of diethylstilbestrol (17a, 17b). Steiner and Kendall (18) used this fact in later studies along the same lines. They fed cholesterol plus thiouracil (a thyroid-inhibiting drug) to dogs and this time succeeded not only in producing hypercholesterolemia, but arterial lesions similar in distribution and morphology to those seen in human atherosclerosis. Feeding thiouracil without cholesterol did not cause arteriosclerosis.

†*cf. Borden's Review of Nutrition Research,* February, 1949, "Dietotherapy in Hypertension."

The significance of hypercholesterolemia, whatever its cause, as a direct factor in the development of human atherosclerosis is in even greater dispute. While it is apparent that the two are related, it has not yet been demonstrated that they are necessarily cause and effect, particularly since atherosclerosis often develops in the absence of hyperlipemia or hypercholesterolemia.

Role of Cholesterol in Lesion Formation:

Innumerable theories have been developed in an attempt to explain the mechanisms involved in the development of arteriosclerotic lesions. These have suggested mechanical factors, toxins, disturbances in oxygen supply, and others as likely causes. In the case of atherosclerosis, it is quite evident that hyperlipemia and hypercholesterolemia are in some way related although by no means essential to the ultimate development of arterial lesions. Similarly, the specificity of cholesterol in this picture, has been generally accepted, but it is obviously not cholesterol *per se* which is the direct causative factor.

Modern Concept: In the past few years, a new concept has been developing concerning the significance of these suspiciously related factors in the picture of developing arteriosclerosis. It may be pieced together from the combined contributions of many independent workers. Thus, back in 1942, Hellwig (19) reiterating some of the older theories on the influence of mechanical factors, pointed out the importance of simple physical flocculation. During such a process, small colloidal particles of cholesterol in the plasma may agglomerate, as a result of vibration, into larger particles, analagous to ordinary churning action. This "churning" effect has been used to explain the marked incidence of atheromatoses at certain locations all of which are characterized by constant vibrating motion (as the heart valves and certain portions of the coronary artery). It was believed that these large, aggregate particles lodge in the arterial wall thereby setting up an irritation resulting in the formation of a local lesion. A similar explanation is afforded by the studies of Hueper (20). He was able to produce atherosclerotic lesions in dogs, rabbits and rats by means of large, colloidal particles of foreign substances (polyvinyl alcohol, pectin, methyl cellulose, gum arabic) introduced into the blood stream by injection. He postulated that these large particles interfere with the exchange of gases and nutritive substance through the interface of blood and the intimal layer of the artery thereby causing a thickening of the intima. If these lesions degenerate, he suggested further, they may form atheromas containing the specific cellular debris present (ordinarily cholesterol). He believed that the large molecules of cholesterol

and cholesterol esters could be precipitated out of colloidal dispersion in the blood stream by a simple vibratory action such as described by Hellwig. Consequently, such precipitated molecules form the debris which might interfere with arterial nutrition in the same way as the large molecules of experimentally introduced foreign substances.

The Effect of Chylomicrons: Chylomicrons are large aggregate particles containing chiefly lipids and some cholesterol. They appear in the blood stream after a fat meal and usually remain there for about 5 hours, during which time they disappear gradually as a result of resorptive mechanisms. This is a temporary condition which occurs in all normal individuals and is described as alimentary hyperlipemia. According to Hueper's observations (21, 22) temporary alimentary hyperlipemia and its accompanying high concentrations of chylomicrons would seem to be very similar to the *sustained* hyperlipemia and hyperchylomicronemia of both experimental and pathological origin which are characteristic predisposing factors in atherosclerosis.

This concept of the identity of the physical state of the plasma lipids in both temporary alimentary hyperlipemia (normal) and sustained hyperlipemia (pathological) was confirmed and extended by Moreton (23, 24). He demonstrated that the lipid particles (chylomicrons) in abnormal hyperlipemia are of the same physical size and properties as those observed in alimentary hyperlipemia (3-5 hours following a fatty meal) and that these differ markedly from the small-sized invisible colloidal lipid particles which are found in normal (fasting) plasma. From these observations, coupled with clues from the characteristic histogenesis of the lesion, Moreton suggested the theory that "the cumulative effect of many fatty meals over a lifetime, by producing these transient showers of large lipid particles in the plasma, is the underlying cause of atherosclerosis in normal humans." He points out further that the triglycerides and fatty acids are rapidly resorbed from these mixed-lipid particles, but that cholesterol is difficult to resorb and thus remains behind. This cholesterol accumulates in the intima of the artery where it provokes a defensive foreign body response which results in the formation of a gradually developing lesion.

The Effect of Time and Age: The significance of hyperchylomicronemia has been further elaborated by the studies of Becker and associates (25) on the effect of age on the rate of absorption of dietary fat. They found that in young subjects (average age, 18 years) chylomicron counts reached a peak 2½-3 hours after a fat meal and returned to fasting levels after 5 hours; in older persons (average age, 76 years) peaks were not reached

until 8-12 hours and did not return to fasting levels until after 24 hours.* In addition the total *number* of chylomicrons was consistently greater in the older group. As the authors point out, with increasing age, alimentary chylomicronemia is of greater intensity and overlaps from one meal to another, continually remaining at a high level.

An interesting observation by these workers was the finding that the feeding of lipase (a fat-splitting enzyme) or of a commercial detergent with the fat meal reduced the degree and duration of hyperchylomicronemia in old persons to levels comparable to those of young subjects. No influence of this procedure was noted in young subjects. The prophylactic or therapeutic significance of this treatment cannot be predicted at present, however, without extensive studies on animals.

Specificity of the Macromolecules: A new aspect to the concept of large particles as provoking agents in atherosclerosis was introduced early this year by the significant studies of Gofman *et al* (26). They found that the bulk of dietary cholesterol shows up after feeding in the *lipoproteins* of the serum rather than in the chylomicrons. They investigated these giant molecules (composed of cholesterol, its esters, fatty acids, lecithin and protein) on the premise that a defect might exist in one class of these molecules. Such a defective molecule rather than the total level of serum cholesterol might be responsible in some way for the development of atherosclerosis. Their studies were conducted on both rabbits and human subjects.

(Animal Studies): Physico-chemical studies with the ultracentrifuge show that normal rabbits have a specific serum lipoprotein component which can be identified by its density (1.03 g./ml.) and which has a flotation rate between 5-8 Svedburg units (expressed as S_f5-8). This lipoprotein contains about 30% cholesterol by weight. On feeding rabbits cholesterol, the concentration of this S_f5-8 lipoprotein increases, sometimes as much as fourfold. In some rabbits, however, cholesterol feeding is followed not only by this increase but also by the appearance of a series of *new* cholesterol-bearing giant molecules of lower density, classified as S_f10-30.

Upon autopsy it was found that those rabbits which developed components largely of the S_f5-8 type showed little or no atherosclerosis; mild to severe atherosclerosis developed in those with high concentrations of S_f10-30 components. All groups attained comparable levels of the 5-8 S_f component, thereby eliminating this fraction as the one implicated in atherosclerosis. On the other hand, the correlation between the development of severe atherosclerosis and high concentrations of S_f10-30 components "suggests that at

*All of the subjects were given a standard fat meal of white bread and a fat spread together with a cup of tea and fasted thereafter for 24 hours.

least some of these components either are the molecules which deposit in atheromatous plaques or are a reflection in the blood of the metabolic abnormality which results in cholesterol-induced atherosclerosis."

(Human Studies): Similar examination of human sera samples revealed that several characteristic lipoprotein components may be found, only one of which seems to be universally present and is not affected, over periods of a few days, by meals. This component is characterized as S_f3-8. In certain sera, other components classified as S_f10-20, were also found. These are distinct from the components characteristic of the normal lipemia following meals. The S_f10-20 molecules contain about 30% cholesterol by weight and little or no protein; the S_f3-8 molecules contain about 25% protein by weight.

Marked correlation was found between the presence of S_f10-20 molecules and the development of atherosclerosis. Higher levels of this component were found in older age groups without known arteriosclerosis correlating with the known increase in arteriosclerosis with age. Males from 20-40 years of age without known disease showed correspondingly higher levels than females of the same age group, in agreement with statistical data which show that females of this age group are much less likely to show atherosclerosis than males. About 50% of normal persons over 40 showed an appreciable concentration of the S_f10-20 fraction. This may be compared with known pathological data (derived from autopsies of persons dying of accidental or other causes) which indicate that about one-half of these people are developing arteriosclerosis *even though the disease is not yet clinically evident.*

(Interpretation of Findings): In the rabbit, components analogous to the S_f10-20 class in humans appear as a result of dietary overloading with cholesterol. Gofman *et al* therefore attempted to find whether dietary intake of cholesterol in man would also alter the level of these S_f10-20 molecules. They found that dietary restriction of cholesterol and fat, in 17 out of 20 cases, caused a definite reduction in the number of S_f10-20 molecules over a period of 2-4 weeks. They conclude, therefore, that "exogenous cholesterol in the human as well as in the rabbit is a factor in influencing the blood level of molecules of the S_f10-20 class."

Comparison of total serum cholesterol levels with the concentration of S_f10-20 molecules revealed further interesting facts: Although there is a general tendency toward an increased frequency of such components in sera with cholesterol levels over 200 mg. per cent, this is by no means necessarily so. Many sera with cholesterol levels well below 200 mg. per cent have an appreciable concentration of S_f10-20 molecules. Similarly, many sera with cholesterol values over 200 mg. per cent, were found to be low in the S_f10-20

component. If the conclusions of Gofman *et al* are valid, then the relation between the concentration of S_f10-20 molecules and total serum cholesterol helps explain the confusion in attempts to correlate atherosclerosis with hypercholesteremia (total cholesterol values). Patients with hypercholesteremia (over 300 mg. %) generally show an increase in the S_f10-20 component. However, many persons with normal serum cholesterol levels may have more cholesterol in the S_f10-20 fraction than certain hypercholesteremic persons. In this case, persons with apparently normal cholesterol levels may still have atherosclerosis, without coincident hyperlipemia.

Cholesterol Content of Foods:

Although it is generally agreed that a so-called "low cholesterol diet" is ineffective in reducing blood levels of cholesterol and that, conversely, eating foods rich in cholesterol (within the normal range of eating habits), will not lead to high blood levels of cholesterol, it may be interesting to examine the cholesterol contents of some common foods. It is especially noteworthy to observe that certain foods, commonly regarded as cholesterol-rich when interpreted on the basis of cholesterol content per 100 gram portion, are actually not significant sources of this sterol when given in average servings. This point is illustrated in Table 1 below:

TABLE 1. Comparative Cholesterol Contents of Common Foods (26a)

Food	Mg. per 100 gms.	Mg. per 100 cal.	Mg. per avg. serving
Beef	125	80	125
Brains (beef)	2300	1600	2300
Butter	280	40	40
Cheese, American	160	40	40
Chicken	60–90	40–60	60–90
Codfish	50	50	50
Cow's milk	12	17	28
Eggs, whole	468	330	450
Heart (beef)	2110	1400	2100
Liver (beef)	320	200	320
Pork	70–105	50–70	70–100
Salmon	60	45	60
Shrimp	150	190	100

Vegetables and cereals have zero cholesterol

Conclusion:

Because of the enormous complexity inherent in the circumstances leading to the development of atherosclerosis in man and because of the widely

divergent findings of research investigators it is nearly impossible at this time to formulate a statement concerning the role of one factor, cholesterol, in the etiology of this widespread disease.

As Dock (27) has aptly put it:

> "Whatever the mechanisms may be, the fact is obvious that in human atherosclerosis, fat content of the diet, protein content of the diet, total caloric content and cholesterol content are all important to that group of the population whose plasma cholesterol behaves more like that of the rabbit than of the dog.
>
> "A small group of young people, and far more middle-aged people, have high and unstable cholesterol values, sensitive to added cholesterol or to diets rich in fat and calories. Some others, like dogs, can maintain levels under 200 mg. per cent on rich diets. In many, with intermediate types of metabolism, high calorie, high cholesterol, high fat diets merely provide the straw to break the camel's back — the slight difference in tissue cholesterol level which spells deposition rather than resolution."

In other words, it is not the actual cholesterol content of the diet, but rather the manner in which this substance is handled by the body which will determine its influence on the course and development of arteriosclerosis.

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